Serial No.: 10/039,367
Docket No.: ECV-5608
Amendment dated October 6, 2004
Responsive to Office Action of July 6, 2004

Amendments to the claims:

The following is a complete listing of the claims in the present application:

1. (Currently amended) A method for mitigating post-implantation calcification of a bioprosthetic material, said method comprising the steps of:

(a) heating a glutaraldehyde solution having a pH of between 7.2-7.8 to a first temperature above 20° C. for a first period of time of at least one hour until the pH of the glutaraldehyde solution has been reduced to between 5-7;

~~(b) adjusting the temperature of the glutaraldehyde solution to a second temperature;~~ and,

(c) ~~after the temperature of the glutaraldehyde solution has been adjusted to the second temperature,~~ contacting a quantity of biological tissue that contains connective tissue protein with the pH-reduced glutaraldehyde solution for a second period of time of at least one hour.

2. (Currently amended) A method according to claim 1, wherein the first temperature is maintained for a period of time until the glutaraldehyde solution further exhibits ~~a predetermined end-point is reached, said predetermined end-point being indicated by at least one indicator selected from:~~

a decrease of about 25% or more in the free aldehyde content of the solution;

~~a fall in the pH of the solution from about 7.4 to about 6.0;~~

~~a fall in the pH of the solution by about 20%; and,~~

~~a change in the color of the solution to yellow or brown~~.

3. (Currently amended) A method according to claim 2 wherein the first temperature is no more than about 90° C ~~about 20-90° C~~.

4. (Original) A method according to claim 2 wherein the first temperature is about 60-80° C.

5. (Original) A method according to claim 2 wherein the first temperature is about 70±5° C.

6. (Currently amended) A method according to claim 2 further including the step of:

(b) prior to step (c), adjusting the temperature of the glutaraldehyde solution to a ~~wherein the~~ second temperature ~~is~~ less than the first temperature.

7. (Original) A method according to claim 6 wherein the second temperature is about 30-70° C.

8. (Original) A method according to claim 6 wherein the second temperature is about 40-60° C.

9. (Currently amended) A method according to claim 6 wherein the second temperature is about ~~50±°~~ 50±5° C.

10. (Currently amended) A method according to claim 1 wherein ~~the first temperature is no lower than about 70° C. and~~ the second temperature is ~~no higher than about~~ between about 40-60° C.

11. (Canceled)

12. (Canceled)

13. (Currently amended) A method according to claim 1 wherein the tissue is ~~at least partially~~ fully fixed prior to the performance of Step (c).

14. (Currently amended) A method according to claim 13 ~~1~~ wherein the tissue is fixed by immersing the tissue in a solution of glutaraldehyde for 1-14 days ~~tissue is fixed after the performance of Step (c).~~

15. (Canceled)

16. (Canceled)

17. (Canceled)

18. (Canceled)

19. (Currently amended) A method according to claim 1 wherein the ~~tissue is fixed in the~~ glutaraldehyde solution in Step (c) ~~while the solution~~ is moving relative to the tissue.

20. (Currently amended) A method according to claim 1 wherein the method ~~further~~ comprises:

preparing a solution of 0.1-25% by weight glutaraldehyde;

heating the glutaraldehyde solution to about 20-90° C. in Step (a);

~~adjusting the second temperature to no greater than about 60° C.;~~ and

thereafter immersing the tissue in the glutaraldehyde solution in Step (c) while maintaining the temperature of the solution in the range of about 40° C. to 60° C. for about 1 day to two months.

21. (Withdrawn) A method according to claim 20 further comprising the step of adding any desired non-biological components to the tissue and fabricating a bioprosthesis.

22. (Original) A method according to claim 20 further comprising the step of subjecting the tissue to a bioburden reduction process.

23. (Original) A method according to claim 22 wherein the step of subjecting the tissue to a bioburden reduction process comprises contacting the tissue with a bioburden reduction solution containing a surfactant, an aldehyde and an alcohol.

24. (Original) A method according to claim 23 wherein the bioburden reduction solution comprises:

Formaldehyde 2-10% by weight;

Ethanol 10-45% by weight; and,

Tween 80 (polyoxyethylene (20) sorbitan monooleate) 0.1-10% by weight.

25. (Withdrawn) A method according to claim 20 further comprising the steps of:

adding any desired non-biological components to the tissue and fabricating a bioprosthesis; and,

sterilizing the bioprosthesis.

26. (Withdrawn) A method according to claim 1 further comprising the steps of:

removing the tissue from the heat-treated glutaraldehyde solution;

subjecting the tissue to a first bioburden reduction process;

adding any desired non-biological components to the tissue and fabricating a bioprosthesis;

subjecting the tissue to a second bioburden reduction process; and,

sterilizing the bioprosthesis.

27. (Withdrawn) A method according to claim 1 further comprising the step of sterilizing the tissue.

28. (Withdrawn) A method according to claim 27 wherein the sterilization of the tissue comprises:

contacting the tissue with a terminal sterilization solution and heating said terminal sterilization solution to a temperature between about 20 to 50° C. for a period of time sufficient to ensure the sterility of the bioprosthesis until the time of implantation.

29. (Withdrawn) A method according to claim 28 wherein the sterilization is carried out in a sealed container and further comprises allowing the tissue to remain within said sealed container until the time of implantation.

30. (Withdrawn) A method according to claim 28 wherein the sterilization is carried out in a moving glutaraldehyde solution.

31. (Withdrawn) A method according to claim 28 wherein said terminal sterilization solution comprises an aqueous solution of 0.2-1.0% by weight glutaraldehyde buffered to a pH of approximately 7.4.

32. (Withdrawn) A method according to claim 28 wherein the terminal sterilization solution comprises osmotically balanced salt solution in combination with at least one chemical sterilant.

33. (Withdrawn) A method according to claim 28 wherein the terminal sterilization solution comprises at least one component selected from i) a denaturant, ii) a surfactant, and iii) a crosslinking agent.

34. (Withdrawn) A method according to claim 28 further including the step of:

(b) prior to step (c), adjusting the temperature of the glutaraldehyde solution to a second temperature less than the first temperature, and

wherein the sterilization solution comprises the previously heated and cooled glutaraldehyde solution prepared in Steps (a) and (b) of claim 1 and wherein the tissue treatment of Step (c) is carried out concurrently with the sterilization step of claim 27.

35. (Withdrawn) A method according to claim 1 wherein the tissue is sterilized after Step (c) by an in-container terminal sterilization process comprising the steps of:

providing a container which contains a quantity of a terminal sterilant solution comprising 0.2-1.0% by weight glutaraldehyde buffered to a pH of approximately 7.4;

immersing the tissue in said terminal sterilant solution within said container;

sealing said container;

heating said container, and the terminal sterilant solution and bioprosthesis contained therein, to a temperature of about 37-50° C. for a period of about six hours to six days;

cooling said container, and the terminal sterilant solution and bioprosthesis contained therein, to room temperature; and,

allowing said container to remain sealed until it is desired to implant the bioprosthesis in a mammalian patient.

36-92. (Canceled)

93. (New) A method according to claim 1, wherein the first temperature is maintained for a period of time until the pH of the glutaraldehyde solution has been reduced to 6.0.

94. (New) A method according to claim 93, wherein the pH of the glutaraldehyde solution is initially about 7.4.

95. (New) A method according to claim 1, wherein the first temperature is maintained for a period of time until the pH of the glutaraldehyde solution has been reduced by about 20%.

96. (New) A method according to claim 95, wherein the pH of the glutaraldehyde solution is initially about 7.4.

97. (New) A method according to claim 1 wherein the first period of time is one hour to six months.

98. (New) A method according to claim 97 wherein the first period of time is one day to two months.

99. (New) A method according to claim 97 wherein the first period of time is 1-14 days.

100. (New) A method according to claim 97 wherein the first period of time is 6-8 days.

101. (New) A method according to claim 97 wherein the second period of time is shorter than the first period of time.

102. (New) A method according to claim 97 wherein the second period of time is between 1 to 15 days.

103. (New) A method according to claim 102 wherein the second period of time is between 6 to 8 days.